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(54) Title: A METHOD FOR THE DETECTION AND TREATMENT OF PROSTATE DISEASE

(57) Abstract

The invention generally features a method of diagnosing a precancerous or cancerous condition of the prostate gland by monitoring levels of nerve growth factor receptor, or fragments thereof. The invention also features a method of treating a precancerous of cancerous condition of the prostate gland by administering a preparation of nerve growth factor receptor, or a fragment thereof.

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A METHOD FOR THE DETECTION AND TREATMENT OF PROSTATE DISEASE

Background of the Invention

The invention relates to diagnosis and treatment of prostate disease.

Prostate cancer is the most frequently diagnosed cancer, and the second leading cause of cancer deaths, among men in North America (Moon, T. 1992. *J. Am. Geriat. Soc.* 40:662-627). It is estimated that 30% of men over 50 years of age have latent cases that will remain benign throughout the life span of the individuals. The causes and determinants of progression of prostate cancer are unknown. For treatment of prostate cancer, localized prostate neoplasias are frequently treated by surgery or radiation. Metastatic prostate cancer is often treated with hormonal modulation, using, for example, GnRH analogs or anti-androgens. This provides an average progression-free survival time of 15 months, and an overall survival time of approximately 30 months. The use of chemotherapeutics is limited by its lack of success in patients who are non-responsive to hormonal therapy.

Nerve growth factor receptors are present on the cell surface of many cell types, including sympathetic and CNS neurons, as well as peripheral nerve Schwann cells. NGF interacts with target cells with biphasic equilibrium binding kinetics, reflecting two classes of receptors (Andres et al. 1977. *Proc. Nat. Acad. Sci. USA* 7:2785-2789), termed by Eveleth (1988. *In Vitro Cell. Devel. Biol.* 24:1148-1153) as type I and type II NGF receptors. Type I receptors show a high affinity for NGF ($Kd = 10^{-11}$), whereas the type II receptors show a lesser affinity ($Kd = 10^{-9}$) for NGF (Schecter et al. 1981. *Cell* 24:687-695; Eveleth, *supra*). Studies which have crosslinked labeled NGF to the plasma membrane have

associated the type I receptor with a 140 kD protein (Massague et al. 1981. *J. Biol. Chem.* 256:9419-9424; Kouchalakos et al. 1986. *J. Biol. Chem.* 261:16054-16059), whereas the type II receptor is identified as a 75 kD protein (Green et al. 1986. *J. Biol. Chem.* 261:15316-15326). Approximately 85-90% of the NGF binding sites display characteristics typical of the low affinity p75 receptor (Schecter et al. *supra*).

The high-affinity NGF receptor has been recently identified as trk (Kaplan et al. 1991a. *Science* 252:554-558; Kaplan et al. 1991b. *Nature* 350:158-160; Klein et al. 1991. *Cell* 65:189-197; Ross, 1991. *Cell Regulation* 2:685-690), a proto-oncogene that mediates tyrosine kinase activity. The trk proto-oncogene was originally discovered as a transforming (i.e. cancer-causing) gene isolated from a colon carcinoma biopsy, and has since been well characterized (Coulier et al. 1989. *Mol. Cell. Biol.* 9:15-23; Martin-Zanca et al. 1989. *Mol. Cell. Biol.* 9:24-33). This finding is consistent with the more general observation that a number of members of the tyrosine kinase gene family have been implicated in human cancer (Coulier et al. *supra*).

Summary of the Invention

The invention features a method of diagnosing a precancerous or cancerous condition in a human patient involving measuring the amount of nerve growth factor receptor (NGFR) present in a biological sample of the patient, a level of NGFR below a predetermined level indicating the presence of the precancerous or cancerous condition.

"Nerve Growth Factor Receptor (NGFR)", as used herein, refers to a low affinity p75 protein receptor, i.e., the type II NGF receptor, and fragments thereof, as distinguished from the high affinity protein receptor, which has been identified as a distinct protein trk. The

low affinity p75 protein receptor, hereinafter referred to as NGFR, can be generally identified by its homology to the cloned nucleic acid sequence or predicted amino acid sequence of Chao et al. (1986. *Science* 232:518-521). An NGFR of the invention has the biological activities of binding NGF with a Kd of 1-10 nM for the intact protein, and as high as 42 nM for the recombinant extracellular domain (RED) of NGFR (Vissavajjhala, et al. 1990. *J. Biol. Chem.* 265:4746-4752).

By "predetermined level of NGFR" is meant a level of NGFR that is standardized by repeated NGFR assays, or a level of NGFR established by concurrently run controls with biological samples known to be normal, i.e., free of any cancerous or precancerous condition.

In preferred embodiments, the precancerous or cancerous condition can be present in any biological tissue of the human body, and is best exemplified, but not limited to, disease conditions of the prostate gland. The biological sample can be in a living patient, can be isolated from the patient as a tissue sample, or can be a body fluid, e.g., urine, semen, or plasma.

When the biological sample is in a living patient, i.e., when the method of diagnosis is performed directly on the patient, the measuring can include administering a labeled anti-NGFR antibody, e.g., a humanized monoclonal antibody, to the patient, and determining the amount of the antibody that is proximal to the prostate gland. The amount of antibody can be determined by imaging, e.g., by positron emission tomography (PET) or by single photon emission computed tomography (SPECT). The label used to make the labeled antibody can be, but is not necessarily, a radioisotope.

When the biological sample is isolated from the patient, the method of diagnosing a cancerous or precancerous condition can further include reacting the

sample with a labeled anti-NGFR antibody, and determining the amount of the antibody bound to the NGFR in the sample as an indication of the amount of NGFR present in the sample. The amount of antibody can be determined when the biological sample is fixed and sectioned, for example, by immunohistochemistry. Alternatively, the amount of antibody can be determined when the biological sample is in solution, preferably after the sample has been homogenized or extracted. NGFR in solubilized tissue can be measured by an immunoassay.

When the biological sample is isolated from the patient, the method of diagnosing a cancerous or precancerous condition can further include introducing a labeled nucleic acid probe to the sample, and determining the amount of the probe bound to NGFR-encoding RNA in the sample, as an indication of the amount of NGFR in the sample. The sample can be, but is not of necessity, histologically fixed. The amount of bound probe can be determined by *in situ* hybridization, or by solution hybridization.

When the biological sample is a body fluid, the method of diagnosing a cancerous or precancerous condition can further include reacting the body fluid with a anti-NGFR antibody, and detecting the amount of the antibody bound to the NGFR in the body fluid as an indication of the amount of NGFR present in the body fluid.

The invention also features a method of treating a precancerous or cancerous condition in a human patient, involving administering a substantially pure preparation of NGFR to the patient. The NGFR can preferably be human NGFR, but can be NGFR from any source. The NGFR can be naturally occurring, or a recombinant form of the complete NGFR polypeptide, or can be a biologically active fragment of NGFR, a truncated fragment of NGFR,

i.e., NGFRt, or the recombinant extracellular domain of NGFR, called RED.

A "substantially pure preparation of NGFR" is a preparation which is substantially free of the proteins with which NGFR naturally occurs in a cell.

"Homology" refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomeric subunit, e.g., if a position in each of two polypeptide molecules is occupied by leucine, then the molecules are homologous at that position. The homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences. For example, 6 of 10, of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the amino acid sequences Leu-gly-val-ala-gly-pro and Leu-his-tyr-ala-gly-leu share 50% homology.

Applicants have discovered that the expression of NGFR is decreased in benign prostate hyperplasia tissue and prostatic adenocarcinoma tissue relative to normal prostate tissue, and is totally absent in four metastatic tumor cell lines of the human prostate. Accordingly, this invention permits a rapid means for diagnosing the neoplastic progression of the human prostate. NGFR can be an effective therapeutic when administered to a patient diagnosed with a cancerous or precancerous condition of the prostate gland.

Other features and advantages of the invention will be apparent from the following description and from the claims.

Brief Description of the Drawings

Fig. 1 is a phase contrast image of a primary culture of normal prostate epithelial tissue showing

vesicles (arrow) in the cytoplasm (A), and of the TSU-pr1 human prostate epithelial tumor cell line (E).

Fig. 2 shows an immunoblot analysis of p75 NGFR in microsomal preparations of human prostate adenocarcinoma tissue (PA), normal prostate (NP), renal tissue (RT), A875 human melanoma cell line which overexpresses the p75 NGFR (A8), and testis (T).

Fig. 3 shows an immunoblot analysis of p75 NGFR in microsomal preparations of (A) five prostatic adenocarcinoma (PA_1-PA_5) specimens, showing decreased or absent immunoreactivity of the adenocarcinoma specimens and benign prostatic hyperplasia (BP_1-BP_5) specimens in comparison with normal prostate (NP) tissue.

Fig. 4 shows an immunoblot analysis of p75 NGFR in microsomal preparations of human prostate stroma (HS), human prostate epithelial tumor cell lines, TSU-pr1 (TS), DU-145 (DU), PC-3 (PC) and LNCaP (LN) showing loss of expression of the p75 NGFR in the metastatic epithelial cell lines.

Detailed Description

In the human prostate, a low affinity p75 nerve growth factor receptor (NGFR) localizes to the epithelia while a NGF-like protein localizes to the stroma. This NGF-like protein, derived from prostate stromal cell cultures, has been shown to participate in paracrine mediated growth of a human tumor epithelial cell line (TSU-pr1) *in vitro*. Applicants have examined the expression of the NGFR in normal prostate tissues, benign prostatic hyperplasia (BPH) tissues, adenocarcinoma tissues, and four metastatic tumor cell lines of the human prostate. In so doing, applicants observed expression of the p75 NGFR in normal prostate tissue, partial loss of NGFR expression in benign and malignant prostate tissue, and complete loss of NGFR expression in the four metastatic tumor cell lines. This suggests an

inverse relationship between p75 NGFR expression and neoplastic progression of the human prostate, which is demonstrated by the experiments below.

Applicants then designed diagnostic procedures that make use of the observed inverse relationship between NGFR expression and neoplastic progression for early detection of cancerous or precancerous condition in a human patient. Once the condition is detected, the patient can be treated with a preparation of NGFR. The situation in prostate tissue is provided as an example of how a decrease in NGFR levels can generally be used to indicate neoplastic transformation. The discovery, motivation and methods of the invention are further described as follows.

Methods

Cell Lines and Tissue Samples: PC-3, DU-145 and LNCaP prostate epithelial tumor cell lines were obtained from the American Type Culture Collection (ATCC; Rockville, MD). The TSUpr1 cell line was provided by Dr. John Isaacs (Johns Hopkins University, Baltimore, MD). The human melanoma cell line (A875) was provided by the laboratory of Dr. Moses Chao (Cornell University, New York, NY). The PC-3, DU-145 and TSU-pr1 cell lines were maintained in RPMI 1640 medium supplemented with antibiotics/antimycotic (100 units/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml fungizone), 10% fetal bovine serum (FBS), and 10^{-7} M testosterone (T). The LNCaP cell line was maintained in Dulbecco's Modified Eagle's Medium (DMEM)/10% FBS and the A875 cell line was maintained in RPMI 1640 medium supplemented with 10% FBS. The BPH tissue was obtained from transurethral resections of the prostate; prostatic adenocarcinoma and normal prostate tissue from radical retropubic prostatectomy specimens; renal tissue from radical nephrectomy specimens and testis from orchiectomy specimens at

Georgetown University Medical Center. The human prostatic stromal cells and epithelial cells were isolated by enzymatic digestion of tissue samples in a collagenase solution as previously described (Djakiew et al. 1991. *Canc. Res.* 51:3304-3310). The prostate stromal cells were cultured in 75 cm^2 tissue culture flasks (Costar, Cambridge, MA) maintained in RPMI 1640 medium containing 10% FBS/T. The normal prostate epithelial cells were plated on glass coverslips and maintained in RPMI 1640 medium containing a serum free defined medium (SFDM) consisting of 2 μg/ml insulin, 10 ng/ml epidermal growth factor, 1 μg/ml transferrin, 10^{-7} M dexamethasone, 2 mM glutamine, 2.5 mg/ml bovine pituitary extract (UpState Biotechnology Inc., Lake Placid, N.Y.), 10 ng/ml rat prolactin (NIH) and 10 μg/ml cholera toxin, all of which were also supplemented with 10% FBS/T and dihydrotestosterone (10^{-7}). All cell cultures were incubated at 37°C in 5% CO_2/95% air and the media replaced every second day.

Unless described otherwise, media, tissue culture supplies, reagents and chemicals can be purchased from Sigma Chemical Co. (St. Louis, MO).

Immunocytochemistry. Cells were grown on glass coverslips to approximately 50% confluence. The cells were washed twice in phosphate buffered saline (PBS) and incubated in unsupplemented RPMI 1640 media for 3 hours. The media was then replaced with fresh unsupplemented RPMI 1640 media for another 3 hours. Human prostate stromal cell conditioned media was prepared as previously described (Djakiew et al., *supra*) and added at concentrations of 20 μg/ml, 5 μg/ml and 1 μg/ml to cultures of the primary epithelial cells and cell lines for a 24 hour incubation period. Subsequently, the cells were fixed in ice cold methanol for 2.5 minutes, blocked with 3% ovalbumin for one hour at 37°C to reduce non-specific binding, and

incubated with murine anti-human p75 NGFR monoclonal antibody (1:80 dilution; Boehringer Mannheim, Indianapolis, IN) for three hours at 37°C. The coverslips were rinsed with PBS (X6) and incubated in rhodamine conjugated sheep anti-murine IgG secondary antibody (1:400 dilution, Cappel, Durham, NC) for one hour. Murine IgG partially purified from whole mouse serum (Cappel) by the method of Jemmerson and Margoliash (Jemmerson et al. 1981. *Meth. Enzymol.* 74:244-272) was used as a non-specific control. Immunocytochemical staining of cell cultures was visualized with a Zeiss photomicroscope fitted with an epifluorescence attachment.

Immunoblot Analysis. Microsomal and cytosolic fractions of cell lines and tissue specimens were obtained after homogenization and sonication by the modified fractionation method of Culty (Culty. 1984. Thesis. *Phosphoinositide metabolism and steroidogenesis of adrenal cortex*. Grenoble, France: Universite Scientifique et Medicale de Grenoble). Cytosolic fractions were dialyzed against ice cold distilled water and lyophilized. The subcellular fractions were resuspended in 50 mM Tris-HCI (pH 7.4) supplemented with 10 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) and 10 μM phenylmethlysulfonylfluoride (PMSF). Nonreducing sample buffer was added to 50 μg of each microsomal and cytosolic protein sample. Subsequently, the samples were separated by sodium dodecyl sulfate-polyacrylamide gel (10%) electrophoresis (SDS-PAGE). The protein from each sample was electrotransferred onto nitrocellulose and blocked for one hour in 5% nonfat dry milk in TBS (20 mM Tris-HCl, 500 mM NaCl, pH 7.5). The membranes were incubated overnight at room temperature with murine monoclonal anti-human p75 NGFR antibody (1:80 dilution) in TBS supplemented with Tween-20 (TTBS) and 1%

gelatin (Bio-Rad, Richmond, CA). The membranes were rinsed twice for 10 minutes with TTBS and reacted with horseradish peroxidase conjugated goat anti-mouse IgG (1:2000) in TTBS containing 1% gelatin for one hour and rinsed twice in TTBS and once in TBS. Immunoreactivity was visualized using 4-chloro-1-napthol hydrogen peroxide reaction substrate.

Results

Immunofluorescence. Immunocytochemical localization of the p75 NGFR in normal prostate epithelial cells and the TSU-pr1 cell line are shown in Fig. 1. The p75 NGFR was observed in cytoplasmic vesicles of the normal prostate epithelial cells (Fig. 1B). In normal prostate epithelial cells treated with 20 μg/ml human prostatic stromal (hPS) protein (Fig. 1C), there appeared to be a greater number of dispersed NGFR immunostained vesicles than in the untreated cells (Fig. 1B). Substitution of the primary antibody with a murine IgG eliminated p75 NGFR fluorescence (Fig. 1D). The prostate epithelial tumor cell line TSU-pr1 (Fig. 1E) treated with 20 μg/ml hPS (Fig. 1F), or in the absence of hPS (not shown), showed no immunofluorescence when stained for the p75 NGFR. The DU-145, PC-3 and LNCaP cell lines also did not show p75 NGFR immunostaining. The A875 human melanoma cell line, which overexpresses the p75 NGFR, was used as the positive control.

Immunoblot Analysis. Fig. 2 shows the results of an immunoblot with the p75 NGFR antibody of microsomal fractions of normal prostate and adenocarcinoma tissue specimens. A 75 kD immunoreactive protein was evident in the normal prostate tissue. The prostate adenocarcinoma tissue exhibited reduced expression of the NGFR protein. Renal tissue was used as the negative control, whereas testis tissue and the A875 melanoma cell line were used as the positive controls. In order to further examine

reduced NGFR expression in the prostate adenocarcinoma, five separate prostatic adenocarcinoma specimens were examined (Fig. 3A). There was a reduction or absence of expression of the p75 NGFR in all of the adenocarcinoma specimens relative to the normal prostate tissue when samples were loaded on an equal protein (50 μg/lane) basis (Fig. 3A). Similarly, there was a reduced expression of p75 NGFR in all of the BPH tissue specimens relative to the normal prostate tissue when samples were loaded on an equal protein (50 μg/lane) basis (Fig. 3B). Microsomal preparations from the prostate tumor cell lines, TSU-pr1, DU-145, PC-3 and LNCaP showed complete absence of p75 NGFR immunoreactivity (Fig. 4). No immunoreactivity for the p75 NGFR was evident in cytosolic fractions of any of the prostate tissue specimens or cell lines examined.

A Decrease in NGFR expression is Indicative of Neoplastic Transformation in Human Tissues

The finding that progression to malignancy is associated with the loss of the low-affinity NGFR suggests to applicants that the NGFR could function in some cases as a tumor suppressor. Under normal physiological conditions NGF can bind to both the low-affinity NGFR as well as to the higher affinity trk. Typically, the low-affinity NGFR is present on cells in very large excess (Vale et al. 1985. *Meth. in Enzymology* 109:21-39). However, if the low-affinity NGFR is decreased, the interaction of NGF with trk would increase, in turn activating a tyrosine kinase activity in the affected cells. Increased tyrosine kinase activation is associated with cellular transformation (Ullrich et al. 1990. *Cell* 61:203-212). Indeed, in NIH 3T3 cells transfected with the trk proto-oncogene, NGF induced the transformation of these recipient cells (Cordon-Cardo et al. 1991. *Cell* 66:173-183). The transforming activity of the trk proto-oncogene was found

to be similar to that of most oncogenes and just 5- to 10-fold lower than that of trk oncogenes isolated from human tumors. Thus, the loss of the low-affinity NGFR on prostate cells, for example, could greatly increase the potential for trk activation by NGF and the consequent transformation of these cells.

This situation could occur in any tissue where the balance of low- and high-affinity NGF receptors is disturbed by a decrease in the low-affinity NGFR.

Methods for Diagnosing a Cancerous or Precancerous Condition

All of the diagnostic methods of the invention are designed to detect and quantitate the amount of NGFR in a tissue. The diagnostic methods of the invention can be used on a living patient, on tissue that has been removed from the patient, or on bodily fluids. The amount of NGFR measured in the tissue, or in the patient, is compared to the amount of NGFR present in normal tissue. The amount of NGFR present in normal tissue can be measured side-by-side with experimental tissue, or, if possible, a standard level of NGFR can be established, where it is shown by repetitive measurements that the level of NGFR routinely falls within an expected concentration range.

The diagnostic methods of the invention are carried out as follows:

Example 1

For measurement of a decrease in NGFR in the patient, an anti-NGFR antibody, preferably a humanized monoclonal antibody, is labeled with a suitable radioisotope for imaging, and is administered to the patient. The amount of radioactive uptake is then determined in the region of the tissue being examined, e.g., in the region of the prostate gland. Imaging is carried out by positron emission tomography (PET), single photon emission computed tomography (SPECT), or other

imaging techniques that can accurately measure radio-labeled antibody.

Anti-NGFR antibodies useful for the diagnostic methods of the invention can include any polyclonal or monoclonal antibody that binds specifically to NGFR, or to a fragment of NGFR, e.g., NGFRt. One example of such an antibody is the monoclonal antibody 8211 (Eveleth, D.D. 1988. *In Vitro Cell Develop. Biol.* 24:1148; Herlyn, M. et al. 1983. *Cancer Invest* 1:215) which is commercially available from Boehringer Mannheim (Indianapolis, IN). Antibody 8211 was used above to measure a reduction in NGFR in precancerous and cancerous prostate cells. Other suitable antibodies include those disclosed by Ross et al. (U.S. 4,786,593, hereby incorporated by reference), Johnson (U.S. 4,855,241, hereby incorporated by reference), and DiStefano and Clagett-Dame (WO 92/09631, hereby incorporated by reference). These references also disclose methods that can be useful for the generation of other suitable antibodies.

The antibody is labeled with a suitable detectable element, e.g., a radioisotope. For example, the antibody can be chemically radiolabelled with Iodine-123 or Technecium 99m, either of which is useful for SPECT imaging. The antibody can also be labelled with non-radioactive labels, e.g., a paramagnetic ion. Some suitable radioactive and non-radioactive labels are disclosed in WO 91/01144, as are several of many possible methods for attaching said labels to proteins. After it is labeled, the antibody can be detected in the human body by PET, SPECT, or Nuclear Magnetic Resonance (NMR) imaging, or another imaging method most suitable for the particular label on the antibody, using techniques which are known to those skilled in the art. A reduction in uptake of the labeled antibody is diagnostic of the

presence of a precancerous or cancerous condition in the tissue being examined.

Example 2

For measurement of a decrease in prostatic NGFR in a tissue sample from a patient, the tissue sample is suitably fixed and sectioned, and the tissue section reacted with anti-NGFR antibody to immunohistochemically determine the amount of NGFR. For this test the tissue sample is removed from the patient, fixed, stained, and counterstained as described by Ross et al. (U.S. 4,786,593, hereby incorporated by reference). Any method of preparing the tissue for immunohistochemistry can be employed that permits the maintenance and visual detection of the NGFR antigen in a tissue section. Such methods are well-known to those skilled in the art. A reduction in staining intensity, relative to normal tissue, is diagnostic of the presence of precancerous or cancerous cells. Immunostaining can be carried out using an NGFR antibody disclosed above.

Example 3

In an additional method for measurement of a decrease in prostatic NGFR in a tissue sample from a patient, the tissue is suspended in solution. The tissue sample is suitably homogenized to extract NGFR by any of a number of methods known to those skilled in the art that permit the maintenance of the NGFR antigen. The level of NGFR is determined by immunoassay. Quantitation of the antigen is carried out by using an NGFR antibody disclosed above. The particular format of immunoassay can be any of several types of enzyme immunoassay, as described (Tijssen, P., *Practice and Theory of Enzyme Immunoassays*, Elsevier 1985) or radioimmunoassay (Chard, T., *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier, 1982), the most effective versions of which are known to those skilled in the art. A

reduction in tissue concentrations of NGFR antigen, relative to normal prostatic tissue, is diagnostic of the presence of precancerous or cancerous cells.

Example 4

For measurement of a decrease in NGFR expression in a tissue sample from a patient, the tissue sample is suitably fixed and sectioned, and reacted with a labeled nucleic acid probe to determine, by *in situ* hybridization, the amount of NGFR mRNA. For this test, the tissue sample is frozen, sectioned, and reacted with an NGFR nucleic acid probe, as described by Springer et al. (1990. *Cell. Mol. Neurobiol.* 10:33-39; 1991. *J. Histochem. Cytochem.* 39:231-234). These methods provide for the autoradiographic or enzyme histochemical detection of NGFR mRNA, respectively, in a tissue section. A reduction in label or staining intensity, relative to normal tissue, is diagnostic of the presence of precancerous or cancerous cells. *In situ* hybridization histochemical detection of NGFR can be carried out using any nucleic acid probe, whether chemically or biologically synthesized, that is complementary to NGFR mRNA and labeled by methods that will enable the detection of the probe. The most effective methods of nucleic acid detection are standard, and known to those skilled in the art.

Example 5

For measurement of a decrease in NGFR expression in a tissue sample from a patient, the tissue sample can be suitably homogenized and extracted, and the level of NGFR mRNA determined by a suitable hybridization assay, e.g. a dot blot, Northern blot, polymerase chain reaction (PCR), or solution hybridization assay. For this test, the tissue sample is suitably homogenized and the RNA extracted by methods that are known to those skilled in the art. Suitable nonradioactive hybridization assays

have been disclosed (Guesdon, J.L., 1992, *J. Immunol. Methods* 150:33-49; Kricka, L.J., ed., Nonisotopic DNA Probe Techniques, Academic Press, 1992). Alternatively, radioactive methods, which are well known to those skilled in the art, may also be employed, for example, using solution hybridization (Buck, et al. 1988. *Dev. Brain Res.* 44:259-268). Any nucleic acid probe, substantially complementary to NGFR mRNA, of suitable length, and appropriately labeled for detection using the above methods can be used. One example of a suitable probe is provided in Springer et al. 1990, 1991 *supra*. A reduction in hybridization intensity (i.e., in NGFR mRNA), relative to normal tissue, is diagnostic of the presence of precancerous or cancerous cells.

Example 6

For measurement of a decrease in NGFRt in a body fluid sample from a patient the sample is first obtained from the patient. Preferred body fluids include urine, seminal fluid, blood, plasma, mucous, or any other fluid that can be collected from a human patient. The level of NGFRt is determined by immunoassay or NGF ligand binding assay. For this test, the body fluid is assayed for the presence of NGFRt using an immunoassay as disclosed by Johnson (U.S. 4,855,241, *supra*) or DiStefano and Clagett-Dame (WO 92/09631, *supra*) or another suitable immunoassay as referenced above. Alternatively, the body fluid sample is assayed for the presence of NGFRt using a ligand binding assay with either radio-iodinated NGF or biotinylated NGF, followed by suitable precipitation and detection methods, as are known to those skilled in the art. A reduction in ligand binding to NGFRt in the body fluid, relative to the normal level in the fluid, is diagnostic of the presence of precancerous or cancerous cells.

Therapy

The loss of expression of NGFR in prostatic tissue is clearly linked to neoplastic progression. A suitable therapeutic treatment for a patient diagnosed with a reduction in NGFR or NGFRt is systemic administration of a recombinant form of a human NGFRt, which has the effect of substituting for the loss in prostatic NGFR as a binding compartment for endogenous NGF. This polypeptide, also referred to as the recombinant extracellular domain (RED) of the NGFR, has been cloned and expressed (Vissavajjhala, P. et al. 1990. *J. Biol. Chem.* 265:4746-4752). Other methods for expressing recombinant human proteins are well known (e.g., Goeddel, D.V., ed. Methods in Enzymology, vol. 185, Academic Press, 1990, hereby incorporated by reference).

Preparation of Substantially Pure NGFR protein

Methods for the purification of substantially pure NGFR are described by Vassavajjhala and Ross (Vissavajjhala et al. *supra*). In addition, with the availability of the cloned gene, a substantially pure NGFR polypeptide can be produced in quantity using standard techniques known to one skilled in the art (see, e.g., Scopes, R. Protein Purification: Principles and Practice, 1982 Springer Verlag, NY). For example, the NGFR protein or fragment can be purified using conventional methods of protein isolation known to one schooled in the art, e.g., methods including but not limited to precipitation, chromatography, immunoadsorption, or affinity techniques. The polypeptide can be purified from starting material using the cloned NGFR gene, or using a recombinant form of the NGFR DNA or cDNAs genetically engineered into an overproducing cell line.

Methods of Administration

The NGFR preparation can be administered to a human patient in a pharmaceutically acceptable buffer (e.g., physiological saline, which may also contain stabilizing agents as excipients). The therapeutic NGFR preparation is administered in accordance with the location and condition of the neoplasia to be treated. For example, the therapeutic preparation can be administered systemically, orally, parenterally, transdermally, or transmucosally. Administration can be in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery using micelles, gels or liposomes, or by transgenic modes. Preferably, the NGFR preparation is administered systemically, e.g., by an intravenous, subcutaneous, or intramuscular injection. Alternatively, it may be necessary to administer the targeted virus surgically to the appropriate target tissue, or via a catheter, or a videoscope.

An appropriate therapeutic dose is an amount of NGFR which effects a reduction in, or postpones progression of, a neoplasia. The dosage can be, but is not necessarily, in the range of 0.001 - 100.0 mg/kg body weight, or a range that is clinically determined as appropriate by those skilled in the art.

Other Embodiments

Other embodiments are within the following claims. For example, the methods of the invention can be carried out by administering or detecting any protein that is substantially homologous to a NGFR protein. Also included are: allelic variations; natural mutants; induced mutants; proteins encoded by DNA that hybridizes under high or low stringency conditions (e.g., washing at 2xSSC at 40 °C with a probe length of at least 40

nucleotides) to a naturally occurring NGFR nucleic acid (for other definitions of high and low stringency see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989, 6.3.1 - 6.3.6, hereby incorporated by reference); and polypeptides or proteins specifically bound by antisera to a NGFR protein, especially by antisera to the active site or binding domain of a NGFR protein. The term also includes chimeric polypeptides that include biologically active fragments of the NGFR protein.

The invention also includes any biologically active fragment or analog of a NGFR protein. By "biologically active" is meant possessing *in vivo* or *in vitro* receptor binding activity which is characteristic of the NGFR molecule described in Zupan et al. (1989. *J. Biol. Chem.* 264:11714-11720). Since a NGFR protein receptor exhibits a range of physiological properties and since such properties may be attributable to different portions of the NGFR molecule, a useful NGFR fragment or NGFR analog is one that exhibits a biological activity in any biological assay for NGFR activity, as described above. This includes natural or induced proteins with significant affinity for NGF, i.e., those with a Kd of less than 10 μM. An example of such a natural protein is α-macroglobulin (Koo et al. 1989. *Journal of Neuroscience Research* 2:247-261), while an example of an induced protein is a monoclonal antibody to NGF. These proteins, as well as active fragments thereof, are within the scope of the invention since they can functionally mimic the effects of an NGFR administered to a patient.

Most preferably a NGFR protein fragment or analog possesses 10%, preferably 40%, or at least 90% of the activity of a member of the NGFR protein family, in any *in vivo* or *in vitro* NGFR activity assay.

Preferred analogs include NGFR (or biologically active fragments thereof) whose sequences differ from the wild-type sequence only by conservative amino acid substitutions, for example, substitution of one amino acid for another with similar characteristics (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish the polypeptide's biological activity.

Other useful modifications include those which increase peptide stability. Such analogs may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) or D-amino acids in the peptide sequence.

Analogs can differ from a naturally occurring member of the NGFR protein family in amino acid sequence or in ways that do not involve sequence, or in both. Analogs of the invention will generally exhibit at least 70%, more preferably 80%, more preferably 90%, and most preferably 95% or even 99%, homology with a segment of 20 amino acid residues, preferably more than 40 amino acid residues, or more preferably the entire sequence of a naturally occurring NGFR polypeptide.

Alterations in primary sequence include genetic variants, both natural and induced. Also included are analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids. Alternatively, increased stability may be conferred by cyclizing the peptide molecule, or by exposing the polypeptide to phosphorylation-altering enzymes, e.g., kinases or phosphatases. Other useful modifications also include *in vivo* or *in vitro* chemical derivatization of polypeptides, e.g., acetylation, methylation, phosphorylation, carboxylation, or

glycosylation; glycosylation can be modified, e.g., by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps, e.g., by exposing the polypeptide to glycosylation affecting enzymes derived from cells that normally provide such processing, e.g., mammalian glycosylation enzymes; phosphorylation can be modified by exposing the polypeptide to phosphorylation-altering enzymes, e.g., kinases or phosphatases.

In addition to substantially full-length NGFR polypeptides, the invention also includes biologically active fragments of the NGFR polypeptides. As used herein, the term "fragment", as applied to a polypeptide, will ordinarily be at least about 20 residues, more typically at least about 40 residues, or preferably at least about 60 residues in length. Fragments of a NGFR polypeptide can be generated by methods known to those skilled in the art. The ability of a candidate fragment to exhibit a biological activity of a NGFR protein can be assessed by methods known to those skilled in the art as described herein. Also included are NGFR polypeptides containing residues that are not required for biological activity of the peptide, or that result from alternative mRNA splicing or alternative protein processing events.

What is claimed is:

CLAIMS

1. A method of diagnosing a precancerous or cancerous condition in a human patient, comprising

measuring the amount of nerve growth factor receptor (NGFR) present in a biological sample obtained from said patient, a level of NGFR below a predetermined level indicating the presence of said precancerous or cancerous condition.

2. The method of claim 1, wherein said precancerous or cancerous condition involves the prostate gland.

3. The method of claim 1, wherein said method comprises

reacting said sample with a labeled anti-NGFR antibody, and

determining the amount of said antibody bound to said NGFR in said sample as an indication of the amount of NGFR present in said sample.

4. The method of claim 1, wherein said method comprises

contacting a nucleic acid probe with said sample, and

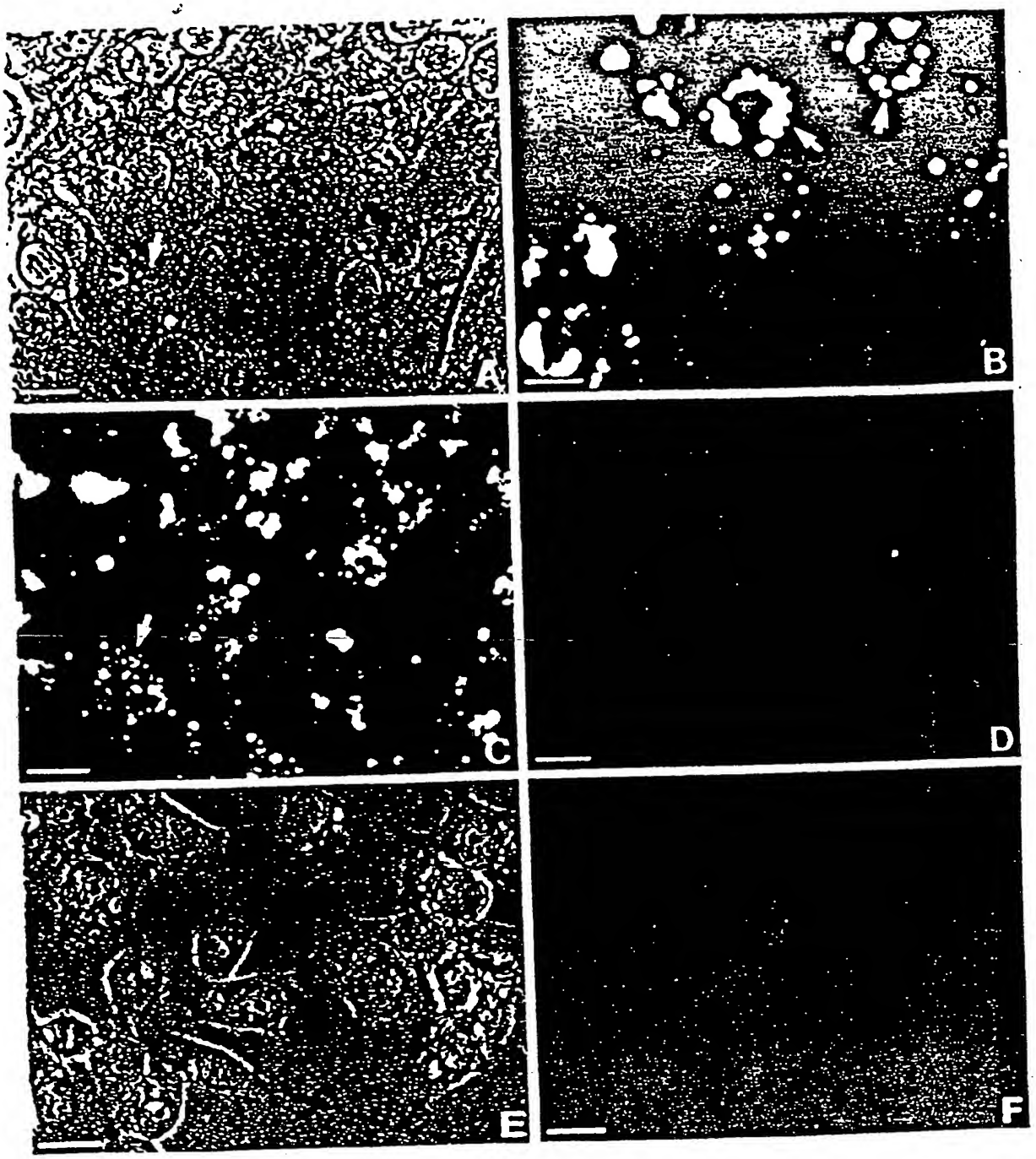
determining the amount of said probe bound to NGFR-encoding RNA in said sample, as an indication of the amount of NGFR in said sample.

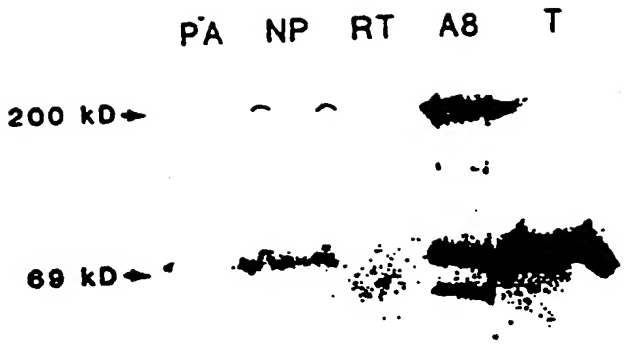
5. Use of a substantially pure preparation of Nerve Growth Factor Receptor (NGFR) or a biologically active fragment thereof in the preparation of a medicament for treating a precancerous or cancerous condition in a human patient.

6. The use of claim 5, wherein said NGFR is human NGFR.

7. Use of an antibody to NGFR in the preparation of a diagnostic for a cancerous or precancerous condition in a human patient.

8. Use of a nucleic acid probe specific for an NGFR coding sequence in the preparation of a diagnostic for a cancerous or precancerous condition in a human patient.

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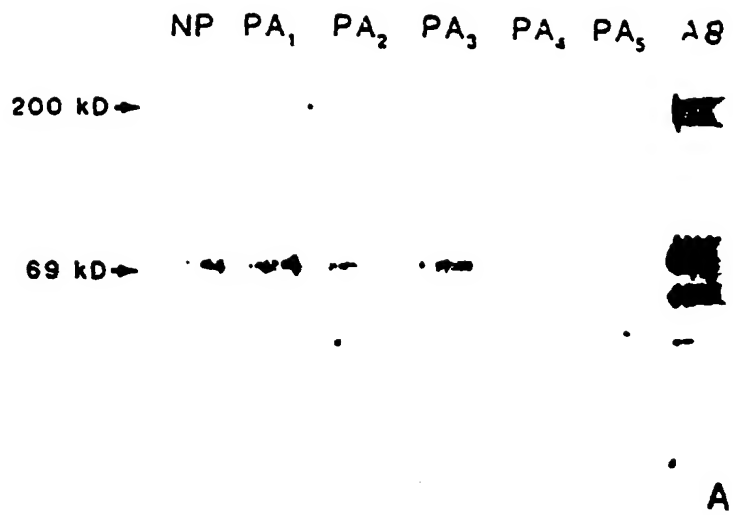

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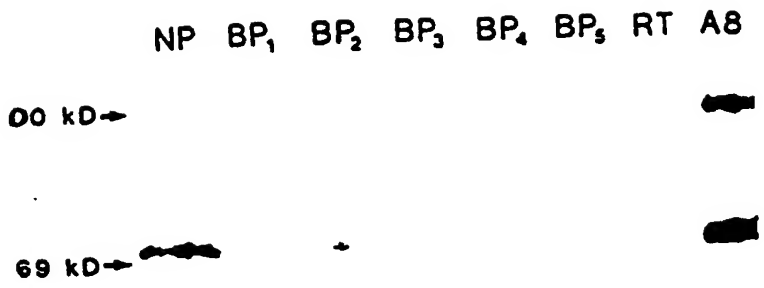
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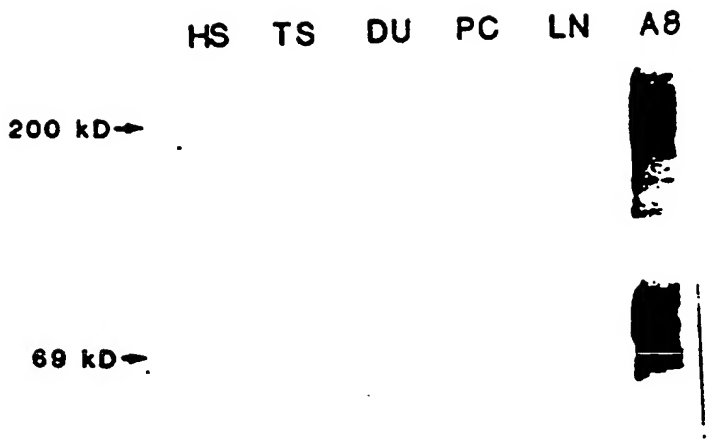
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08446

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68; G01N 33/53
US CL :435/6, 7.1, 7.23, 813
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.23, 813

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, APS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,782,015 (ALLISON ET AL.) 01 NOVEMBER 1988, SEE SUMMARY OF INVENTION AND CLAIMS 1-8.	1-3,5-7
Y	JOURNAL OF UROLOGY, VOLUME 147, ISSUED MAY 1992, GRAHAM ET AL, "DISTRIBUTION OF NERVE GROWTH FACTOR-LIKE PROTEIN AND NERVE GROWTH FACTOR RECEPTOR IN HUMAN BENIGN PROSTATIC HYPERPLASIA AND PROSTATIC ADENOCARCINOMA", PAGES 1444-1447, SEE ENTIRE DOCUMENT.	1-3,5-7
Y	JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, VOLUME 250, NO. 5, ISSUED 05 AUGUST 1983, GOLDENBERG ET AL, "RADIOIMMUNODETECTION OF PROSTATIC CANCER", PAGES 630-635, SEE PAGES 630-633.	1-3

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 05 OCTOBER 1993	Date of mailing of the international search report 02 NOV 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer EGGERTON CAMPBELL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08446

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AMERICAN JOURNAL OF CLINICAL PATHOLOGY, VOLUME 92, NO. 4, ISSUED OCTOBER 1989, THOMPSON ET AL, "A MONOCLONAL ANTIBODY AGAINST NERVE GROWTH FACTOR RECEPTOR", PAGES 415-423, SEE ENTIRE DOCUMENT.	1-3,5-7
Y	CELL, VOLUME 47, ISSUED 21 NOVEMBER 1986, JOHNSON ET AL, "EXPRESSION AND STRUCTURE OF THE HUMAN NGF RECEPTOR", PAGES 545-554, SEE ENTIRE DOCUMENT.	1-8